10/551296

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Benzofuro-1,4-diazepin-2-one derivatives

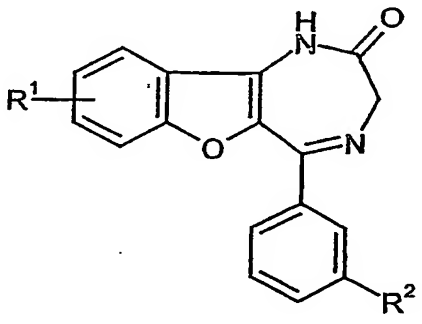
The present invention relates to novel benzofuro-1,4-diazepin-2-one derivatives, process for their preparation and their use as $P2X_4$ receptor antagonists for producing medicaments for the treatment and/or prophylaxis of diseases, in particular of arteriosclerosis, restenosis and other inflammatory disorders.

Arteriosclerosis is a multifactorial disorder whose development is influenced by many different factors. Inflammatory processes inter alia play a central part in this, with inflammation-inducing cytokines such as CD40L and IFNγ being involved [P. Libby, *Nature* 420 (6917): 868-74 (2002)]. The purinergic receptor $P2X_4$ belongs to the P2X family. To date, six different P2X receptors have been described in humans. They take the form of calcium-permeable channels which can be activated by ATP [F. Di Virgilio et al., *Blood* 97 (3): 587-600 (2001); R.A. North, A. Surprenant, *Annu. Rev. Pharmacol. Toxicol.* 40: 563-80 (2000)]. It has been possible to show that there is high-level expression of the $P2X_4$ channel in highly vascularized organs and vessels [K. Yamamoto et al., *Circ. Res.* 87(5): 385-91 (2000)]. Surprisingly, the $P2X_4$ receptor is also expressed on human monocytes. A five-fold increase in $P2X_4$ expression was observable on incubation of human monocytes with CD40L and IFNγ. A high level of expression of the $P2X_4$ receptor has also been found in the vessel wall of the aorta of rabbits after damage by balloon angioplasty and cholesterol feeding [T.J. Pulvirenti et al., *J. Neurocytol.* 29 (9): 623-31 (2000)] and in the arteriosclerotically altered vessel segments of the apoE knockout mouse. Since activated monocytes assume a key function in the early stage of atherogenesis and in restenosis, and monocytes are activated by said cytokines, inhibition of the activation leads to reduction in atherogenesis [P. Libby, *Nature* 420 (6917): 868-74 (2002)]. Since evidently monocyte activation by CD40L and IFNγ is associated via the increase in $P2X_4$ receptor expression and the increased calcium influx connected thereto, blockade of $P2X_4$ receptors ought to reduce inflammatory processes [F. Di Virgilio, A. Solini, *Br. J. Pharmacol.* 135 (4): 831-42 (2002)]. Thus, diseases in which inflammatory processes are involved might be treatable by blockade of $P2X_4$ receptors.

Besides the indications of arteriosclerosis and restenosis, and their sequelae (stroke, angina pectoris, myocardial infarction, renal failure, impaired perfusion of limbs), treatment of other inflammatory disorders such as, for example, psoriasis and rheumatoid arthritis might thus also be possible via the mechanism mentioned.

The synthesis of some benzofuro[3,2-e]-1,4-diazepin-2-one derivatives is described in J. Heterocyclic Chem. 16, 189-90 (1979) and *ibid.* 20, 1251-1254 (1983). Benzofuro-1,4-diazepine derivatives with an antiulcer effect are disclosed in EP 350 131-A.

The present invention relates to compounds of the general formula (I)



(I),

in which

R^1 is halogen

and

R^2 is hydrogen, halogen, nitro, cyano or a group of the formula $-C(O)-OR^3$, $-C(O)-NR^4R^5$, $-SO_2-OR^3$ or $-SO_2-NR^4R^5$, in which

R^3, R^4 and R^5 are independently of one another hydrogen or (C_1-C_6)-alkyl,

or

R^1 is hydrogen

and

R^2 is halogen, nitro, cyano or a group of the formula $-C(O)-OR^3$, $-C(O)-NR^4R^5$, $-SO_2-OR^3$ or $-SO_2-NR^4R^5$, in which

R^3, R^4 and R^5 are independently of one another hydrogen or (C_1-C_6)-alkyl.

The compounds of the invention may also exist in the form of their salts, solvates and solvates of their salts.

For the purposes of the present invention, the substituents generally have the following meaning:

(C_1-C_6)-alkyl and (C_1-C_4)-alkyl are for the purposes of the invention a straight-chain or branched alkyl radical having respectively 1 to 6 and 1 to 4 carbon atoms. A straight-chain or branched alkyl radical having 1 to 4 carbon atoms is preferred. Preferred examples which may be mentioned are: methyl, ethyl, n-propyl, isopropyl and tert-butyl.

Halogen includes for the purposes of the invention fluorine, chlorine, bromine and iodine. Chlorine or bromine are preferred.

The compounds of the invention may, depending on the substitution pattern, exist in stereoisomeric forms which either are related as image and mirror image (enantiomers) or which are not related as image and mirror image (diastereomers). The invention relates both to the enantiomers or diastereomers and to respective mixtures thereof. The racemic forms can, just like the diastereomers, be separated in a known manner into the stereoisomerically pure constituents.

Certain compounds may moreover exist in tautomeric forms. This is known to the skilled worker, and such compounds are likewise included within the scope of the invention.

The compounds of the invention may also exist as salts. Physiologically acceptable salts are preferred for the purposes of the invention.

Physiologically acceptable salts may be salts of the compounds of the invention with inorganic or organic acids. Preference is given to salts with inorganic acids such as, for example, hydrochloric acid, hydrobromic acid, phosphoric acid or sulphuric acid, or salts with organic carboxylic or sulphonic acids such as, for example, acetic acid, propionic acid, maleic acid, fumaric acid, malic acid, citric acid, tartaric acid, lactic acid, benzoic acid, or methanesulphonic acid, ethanesulphonic acid, benzenesulphonic acid, toluenesulphonic acid or naphthalenedisulphonic acid.

Physiologically acceptable salts may likewise be salts of the compounds of the invention with bases, such as, for example, metal or ammonium salts. Preferred examples are alkali metal salts (e.g. sodium or potassium salts), alkaline earth metal salts (e.g. magnesium or calcium salts), and ammonium salts derived from ammonia or organic amines, such as, for example, ethylamine, di- or triethylamine, ethyldiisopropylamine, monoethanolamine, di- or triethanolamine, dicyclohexylamine, dimethylaminoethanol, dibenzylamine, N-methylmorpholine, dehydroabietylamine, 1-ephenamine, N-methylpiperidine, arginine, lysine, ethylenediamine or 2-phenylethylamine.

The compounds of the invention and their salts may also exist in the form their solvates, especially in the form of their hydrates.

Preferred compounds of the general formula (I) are those in which

R^1 is chlorine or bromine

and

R^2 hydrogen, chlorine, bromine, nitro, cyano or a group of the formula $-C(O)-OR^3$ or $-C(O)-NR^4R^5$, in which

R^3, R^4 and R^5 are independently of one another hydrogen or (C_1-C_4)-alkyl,

or

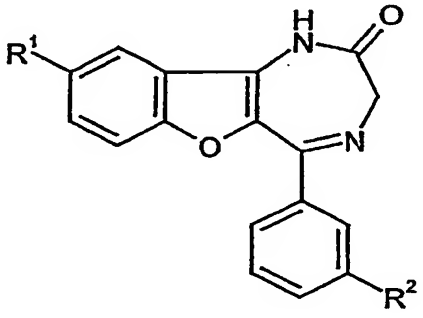
R^1 is hydrogen

and

R^2 is chlorine, bromine, nitro, cyano or a group of the formula -C(O)-OR^3 or -C(O)-NR^4R^5, in which

R^3, R^4 and R^5 are independently of one another hydrogen or (C_1-C_4)-alkyl.

Particularly preferred compounds are those of the general formula (Ia)



(Ia),

in which

R^1 is chlorine or bromine

and

R^2 is hydrogen, chlorine, bromine, nitro or cyano,

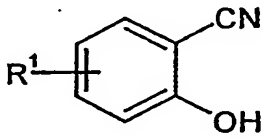
or

R^1 is hydrogen

and

R^2 is chlorine, bromine, nitro or cyano.

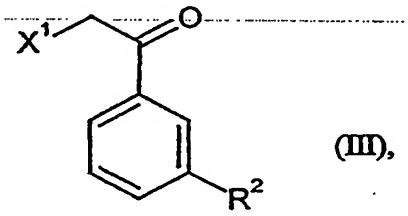
A process for preparing the compounds of the invention has also been found, characterized in that compounds of the formula (II)



(II),

in which R^1 has the meanings indicated above,

are reacted in an inert solvent in the presence of a base with a compound of the formula (III)

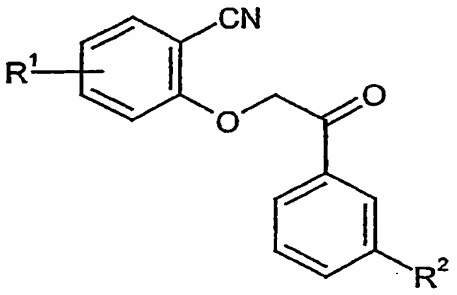


(III),

in which R^2 has the meanings indicated above, and

X^1 is a suitable leaving group such as, for example, chlorine, bromine or iodine,

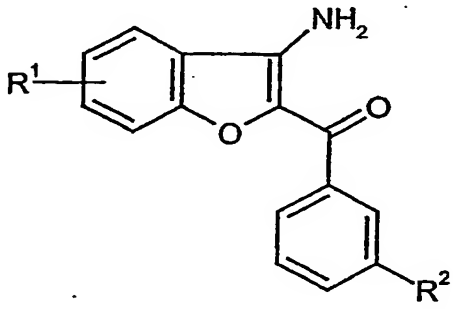
initially to give compounds of the formula (IV)



(IV),

in which R^1 and R^2 have the meanings indicated above,

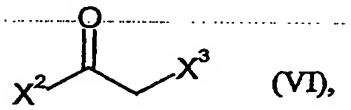
the latter are then cyclized, with intermediate isolation or in a one-pot reaction, in the presence of a base to compounds of the formula (V)



(V),

in which R^1 and R^2 have the meanings indicated above,

subsequently converted in an inert solvent in the presence of a base with a compound of the formula (VI)

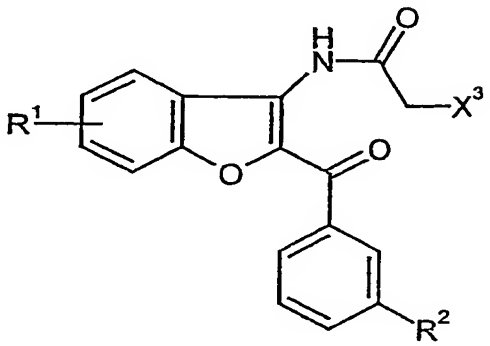


(VI),

in which

X^2 and X^3 are identical or different and are a suitable leaving group such as, for example, chlorine, bromine or iodine,

into compounds of the formula (VII)



(VII),

in which R^1, R^2 and X^3 have the meanings indicated above,

finally reacted with ammonia in an inert solvent for cyclization, and the resulting compounds of the formula (I) are converted where appropriate with the appropriate solvents and/or bases or acids into their solvates, salts and/or solvates of the salts.

Suitable solvents for the process step (II) + (III) → (IV) are inert organic solvents which are not changed under the reaction conditions. These include halohydrocarbons such as dichloromethane, trichloromethane, tetrachloromethane, trichloroethane, tetrachloroethane, 1,2-dichloroethane or trichloroethylene, ethers such as diethyl ether, dioxane, tetrahydrofuran, glycol dimethyl ether or diethylene glycol dimethyl ether, hydrocarbons such as benzene, xylene, toluene, hexane, cyclohexane or petroleum fractions, esters such as ethyl acetate, ketones such as acetone or 2-butanone, heteroaromatics such as pyridine, amides such as dimethylformamide, dialkyl sulphoxides such as dimethyl sulphoxide, or nitriles such as acetonitrile. It is likewise possible to employ mixtures of said solvents. Dimethylformamide is preferred.

The usual inorganic or organic bases are suitable as base for the process step (II) + (III) → (IV). These preferably include alkali metal or alkaline earth metal carbonates such as sodium, potassium or calcium carbonate, alkali metal hydrides such as sodium hydride, amides such as lithium bis(trimethylsilyl)amide or lithium diisopropylamide, or organic amines such as pyridine, 4-*N*,*N*-dimethylaminopyridine, 4-pyrrolidinopyridine, triethylamine, ethyldiisopropylamine, *N*-methylmorpholine, *N*-methylpiperidine, 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Triethylamine is particularly preferred.

The base is employed in this case in an amount of from 1 to 5 mol, preferably in an amount of from 1 to 2 mol, based on 1 mol of the compound of the formula (II).

The reaction generally takes place in a temperature range from 0°C to +150°C, preferably in a temperature range from +20°C to +100°C. The reaction can be carried out under atmospheric, elevated or reduced pressure (e.g. from 0.5 to 5 bar). It is generally carried out under atmospheric pressure.

Suitable solvents for the process step (IV) → (V) are likewise inert organic solvents which are not changed under the reaction conditions. These include halohydrocarbons such as dichloromethane, trichloromethane, tetrachloromethane, trichloroethane, tetrachloroethane, 1,2-dichloroethane or trichloroethylene, ethers such as diethyl ether, dioxane, tetrahydrofuran, glycol dimethyl ether or diethylene glycol dimethyl ether, alcohols such as methanol, ethanol, n-propanol, isopropanol, n-butanol or tert-butanol, hydrocarbons such as benzene, xylene, toluene, hexane, cyclohexane or petroleum fractions, ketones such as acetone or 2-butanone, heteroaromatics such as pyridine, amides such as dimethylformamide, dialkyl sulphoxides such as dimethyl sulphoxide, or nitriles such as acetonitrile. It is likewise possible to employ mixtures of said solvents. Methanol and ethanol are preferred.

The usual inorganic or organic bases are suitable as base for the process step (IV) → (V). These preferably include alkali metal or alkaline earth metal carbonates such as sodium, potassium or calcium carbonate, alkali metal hydroxides such as lithium, sodium or potassium hydroxide, alkali metal alcoholates such as sodium or potassium methanolate, sodium or potassium ethanolate or potassium tert-butoxide, alkali metal hydrides such as sodium hydride, amides such as lithium bis(trimethylsilyl)amide or lithium diisopropylamide, or organic amines such as pyridine, 4-*N,N*-dimethylaminopyridine, 4-pyrrolidinopyridine, triethylamine, ethyldiisopropylamine, *N*-methylmorpholine, *N*-methylpiperidine, 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Sodium methanolate and sodium ethanolate are particularly preferred.

The base is employed in this case in an amount of from 0.5 to 5 mol, preferably in an amount of from 1 to 2 mol, based on 1 mol of the compound of the formula (IV).

The reaction generally takes place in a temperature range from 0°C to +120°C, preferably in a temperature range from +20°C to +100°C. The reaction can be carried out under atmospheric, elevated or reduced pressure (e.g. from 0.5 to 5 bar). It is generally carried out under atmospheric pressure.

Suitable solvents for the process step (V) + (VI) → (VII) are all inert organic solvents which are not changed under the reaction conditions. These include halohydrocarbons such as dichloromethane, trichloromethane, tetrachloromethane, trichloroethane, tetrachloroethane, 1,2-dichloroethane or trichloroethylene, ethers such as diethyl ether, dioxane, tetrahydrofuran, glycol dimethyl ether or diethylene glycol dimethyl ether, hydrocarbons such as benzene, xylene, toluene, hexane, cyclohexane or petroleum fractions, esters such as ethyl acetate, ketones such as acetone or 2-butanone, amides such as dimethylformamide, dialkyl sulphoxides such as dimethyl sulphoxide, or nitriles such as acetonitrile. It is likewise possible to employ mixtures of said solvents. Dichloromethane and trichloromethane are preferred.

The usual inorganic or organic bases are suitable as base for the process step (V) + (VI) → (VII). These preferably include alkali metal or alkaline earth metal carbonates and bicarbonates such as sodium, potassium or calcium carbonate and sodium or potassium bicarbonate, alkali metal hydrides such as sodium hydride, amides such as lithium bis(trimethylsilyl)amide or lithium diisopropylamide, or organic amines such as pyridine, 4-*N*,*N*-dimethylaminopyridine, 4-pyrrolidino-pyridine, triethylamine, ethyldiisopropylamine, *N*-methylmorpholine, *N*-methyl-piperidine, 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) or 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU). Sodium bicarbonate is particularly preferred.

The base is employed in this case in an amount of from 1 to 10 mol, preferably in an amount of from 1 to 5 mol, based on 1 mol of the compound of the formula (V).

The reaction generally takes place in a temperature range from -20°C to +50°C, preferably in a temperature range from -20°C to +20°C. The reaction can be carried out under atmospheric, elevated or reduced pressure (e.g. from 0.5 to 5 bar). It is generally carried out under atmospheric pressure.

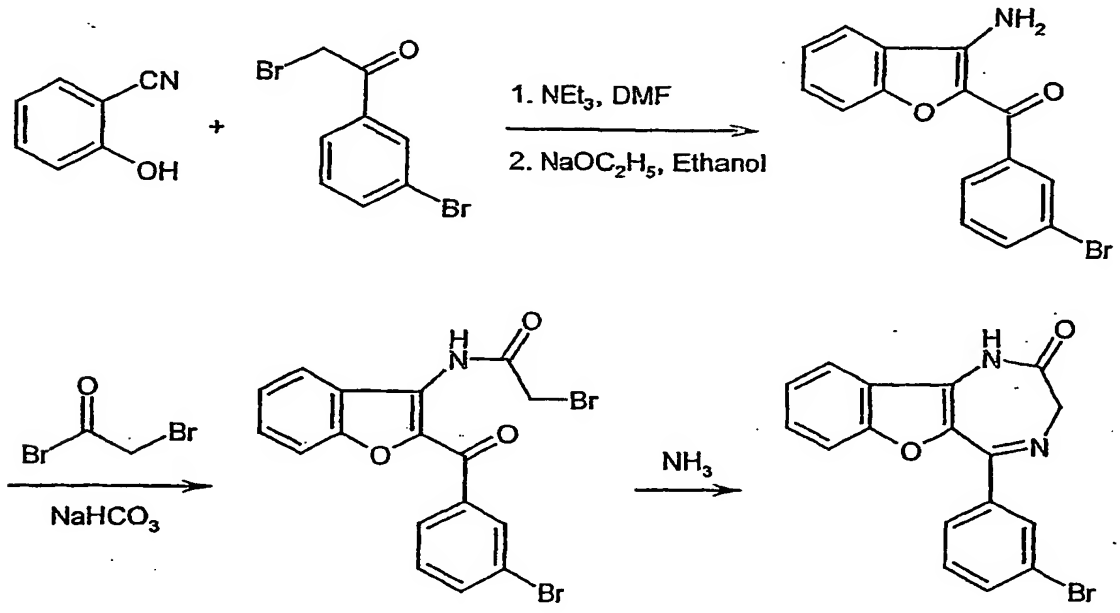
Suitable solvents for the process (VII) → (I) are all inert solvents which are not changed under the reaction conditions. These include halohydrocarbons such as

dichloromethane, trichloromethane, tetrachloromethane, trichloroethane, tetrachloroethane, 1,2-dichloroethane or trichloroethylene, ethers such as diethyl ether, dioxane, tetrahydrofuran, glycol dimethyl ether or diethylene glycol dimethyl ether, or hydrocarbons such as benzene, xylene, toluene, hexane, cyclohexane or petroleum fractions. It is likewise possible to employ mixtures of said solvents. Dioxane is preferred.

The compounds of the formulae (II), (III) and (VI) are commercially available, known from the literature or can be prepared by conventional literature methods [cf., for example, J. Med. Chem. 13, 674-680 (1970)].

The process of the invention can be illustrated by the following formula scheme:

Scheme



The compounds of the invention show a valuable range of pharmacological effects which could not have been predicted and are therefore suitable for use as medicaments for the treatment and/or prophylaxis of diseases in humans and animals.

The compounds of the invention act as antagonists of the $P2X_4$ receptor.

Because of their pharmacological properties, the compounds of the invention can be employed alone or in combination with other medicaments for the treatment and/or prophylaxis of inflammatory disorders. They are particularly suitable for the treatment of chronically inflammatory disorders of the vessel intima such as, for example, arteriosclerosis and restenosis, of inflammatory disorders of the central nervous system such as, for example, multiple sclerosis and pain, of inflammatory disorders of the connective tissue such as, for example, rheumatoid arthritis, chronic polyarthritis, panniculitis and tendinitis, of Bechterew's disease, of inflammatory disorders of the skin such as psoriasis and neurodermatitis, of chronically inflammatory bowel disorders such as enteritis, enterocolitis, Crohn's disease and ulcerative colitis, of inflammatory disorders of the small airways and of myositis and endocarditis.

The compounds of the invention can be administered alone or, if required, in combination with other active ingredients, preferably from the group of CETP inhibitors, antidiabetics, antioxidants, thyroid hormones and/or thyroid mimetics, inhibitors of HMG-CoA reductase, inhibitors of HMG-CoA reductase gene expression, squalene synthase inhibitors, ACAT inhibitors, cholesterol absorption inhibitors, fibrates, MTP inhibitors, triglyceride-lowering agents, nicotinic acid and derivatives thereof, platelet aggregation inhibitors, anticoagulants, calcium antagonists, ACE inhibitors, angiotensin II receptor antagonists, beta blockers and steroidal and nonsteroidal antiinflammatory drugs.

The activity of the compounds of the invention can be tested for example by the tests described in the example section.

The present invention further relates to medicaments which comprise at least one compound of the invention, preferably together with one or more pharmacologically acceptable excipients or carriers, and to the use thereof for the aforementioned purposes.

The compounds of the invention may have systemic and/or local effects. They can for this purpose be administered in a suitable way, such as, for example, by the oral, parenteral, pulmonary, nasal, sublingual, lingual, buccal, rectal, dermal, transdermal, conjunctival or otic route or as implant or stent. Oral administration is preferred.

For these administration routes it is possible to administer the active ingredients in suitable administration forms. Suitable for oral administration are administration forms which function according to the prior art and deliver the active ingredient rapidly and/or in modified fashion, such as, for example, tablets (uncoated and coated tablets, e.g. with coatings which are resistant to gastric juice), capsules, sugar-coated tablets, granules, pellets, powders, emulsions, suspensions and solutions. Parenteral administration can take place with avoidance of an absorption step (e.g. intravenous, intraarterial, intracardiac, intraspinal or intralumbar) or with inclusion of an absorption (e.g. intramuscular, subcutaneous, intracutaneous or intraperitoneal). Administration forms suitable for parenteral administration are, inter alia, preparations for injection and infusion in the form of solutions, suspensions, emulsions, lyophilisates and sterile powders.

Examples suitable for the other administration routes are pharmaceutical forms for inhalation (inter alia powder inhalers, nebulizers), nasal drops/solutions, sprays, tablets or capsules for lingual, sublingual or buccal administration, suppositories, preparations for the ears and eyes, vaginal capsules, aqueous suspensions (lotions, shaking mixtures), lipophilic suspensions, ointments, creams, milk, pastes, dusting powder, implants or stents.

The active ingredients can be converted in a manner known per se into the stated administration forms. This takes place with use of inert nontoxic, pharmaceutically suitable excipients. These include inter alia carriers (e.g. microcrystalline cellulose), solvents (e.g. liquid polyethylene glycols), emulsifiers (e.g. sodium dodecyl sulphate), dispersants (e.g. polyvinylpyrrolidone), synthetic and/or natural biopolymers (e.g. albumin), stabilizers (e.g. antioxidants such as, for example, ascorbic acid), colours (e.g. inorganic pigments such as iron oxides) or taste- and/or odour-masking agents.

It has generally proven advantageous to administer on parenteral administration amounts of about 0.001 to 10 mg/kg, preferably about 0.005 to 3 mg/kg, of body weight to achieve effective results. The amount of oral administration is about 0.001 to 100 mg/kg, preferably about 0.005 to 30 mg/kg, of body weight.

It may nevertheless be necessary where appropriate to deviate from the amounts mentioned, specifically as a function of the body weight, administration route, individual response to the active ingredient, type of preparation and time or interval over which administration takes place. Thus, in some cases it may be sufficient to make do with less than the aforementioned minimum amount, whereas in other cases the stated upper limit must be exceeded. It may in the event of administration of larger amounts be advisable to divide these into a plurality of individual doses over the day.

The percentage data in the following tests and examples are, unless indicated otherwise, percentages by weight; parts are parts by weight. Solvent ratios, dilution ratios and concentration data for liquid-liquid solutions are in each case based on volume.

The following exemplary embodiments illustrate the invention. The invention is not confined to the examples.

Abbreviations:

ICI	chemical ionization (in MS)
DCI	direct chemical ionization (in MS)
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulphoxide
ESI	electrospray ionization (in MS)
LC/MS	coupled liquid chromatography-mass spectroscopy
MS	mass spectroscopy

NMR	nuclear magnetic resonance spectroscopy
R_f	retention index (in TLC)
R_t	retention time (in LC/MS)
TLC	thin layer chromatography

LC/MS methods:

Method 1:

MS instrument type: Micromass ZQ; HPLC instrument type: Waters Alliance 2790; column: Uptisphere C 18, 50 mm × 2.0 mm, 3.0 µm; eluent B: acetonitrile + 0.05% formic acid, eluent A: water + 0.05% formic acid; gradient: 0.0 min 5% B → 2.0 min 40% B → 4.5 min 90% B → 5.5 min 90% B; oven: 45°C; flow rate: 0.0 min 0.75 ml/min → 4.5 min 0.75 ml/min → 5.5 min 1.25 ml/min; UV detection: 210 nm.

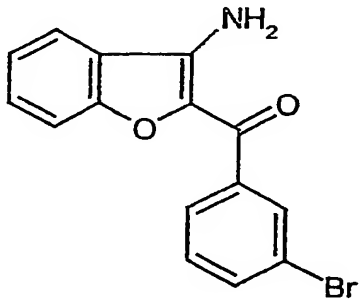
Method 2:

Instrument: Micromass Platform LCZ with HPLC Agilent series 1100; column: Grom-SIL 120 ODS-4 HE, 50 mm × 2.0 mm, 3 µm; eluent A: 1 l of water + 1 ml of 50% formic acid, eluent B: 1 l of acetonitrile + 1 ml of 50% formic acid; gradient: 0.0 min 100% A → 0.2 min 100% A → 2.9 min 30% A → 3.1 min 10% A → 4.5 min 10% A; oven: 55°C; flow rate: 0.8 ml/min; UV detection: 208- 400 nm.

A. Starting compounds:

Example I

(3-Aminobenzofuran-2-yl)-(3-bromophenyl)methanone



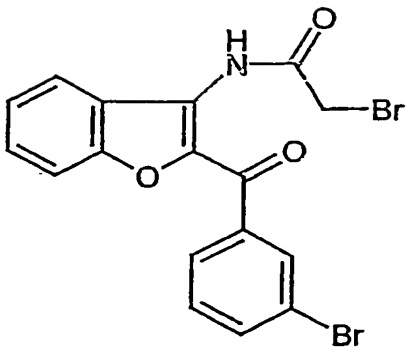
2.00 g (16.8 mmol) of 2-hydroxybenzonitrile, 4.67 g (16.8 mmol) of 3-bromophenacyl bromide and 1.87 g (18.5 mmol) of triethylamine are stirred in 20 ml of dimethylformamide at 70°C for 2 h. After addition of 100 ml of ethyl acetate, the reaction mixture is washed with water (3 × 100 ml) and saturated sodium chloride solution (2 × 100 ml). The organic phase is dried over magnesium sulphate, and the solvent is removed under reduced pressure. The residue is dissolved in 30 ml of ethanol. After addition of 5.98 g (18.5 mmol) of sodium ethanolate, the mixture is heated under reflux for 2 h. 50 ml of ethyl acetate are added, and the mixture is washed with water (3 × 100 ml) and saturated sodium chloride solution (2 × 100 ml). The organic phase is dried over magnesium sulphate. After removal of the solvent under reduced pressure, 5.08 g (92% of theory) of the desired product are obtained.

MS (DCI): m/z = 315.9 $[M+H]^+$

^{1}H-NMR (200 MHz, DMSO-d_6): δ = 7.27-7.36 (m, 1H); 7.49-7.65 (m, 5H); 7.77-7.84 (m, 1H); 8.04-8.20 (m, 3H).

Example II

2-Bromo-*N*-{2-[(3-bromophenyl)carbonyl]benzofuran-3-yl}acetamide



3.53 g (17.5 mmol) of bromoacetyl bromide are added to a mixture of 5.03 g (15.9 mmol) of the compound from Example I and 5.35 g (63.6 mmol) of sodium bicarbonate in 250 ml of chloroform at 0°C. The mixture is stirred at 0°C for 1 h. After removal of the solvent under reduced pressure, 200 ml of ethyl acetate are added, and the mixture is washed with saturated sodium bicarbonate solution (100 ml) and saturated sodium chloride solution (100 ml). The organic phase is dried over magnesium sulphate. After removal of the solvent under reduced pressure, 5.81 g (83% of theory) of the desired product are obtained.

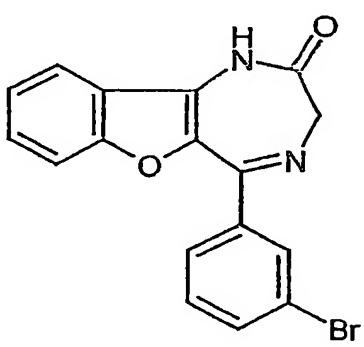
MS (CI): m/z = 436 $[M+H]^+$

^{1}H-NMR (200 MHz, DMSO-d_6): δ = 4.20 (s, 2H); 7.37-8.14 (m, 8H); 10.98 (s, 1H).

B. Exemplary embodiments

Example 1

5-(3-Bromophenyl)-1,3-dihydro-2*H*-benzofuro[3,2-e]-1,4-diazepin-2-one



245 ml (123 mmol) of a 0.5 M solution of ammonia in dioxane are added to 5.37 g (12.3 mmol) of the compound from Example II in 100 ml of diethyl ether. The mixture is stirred at room temperature for 2 days. 100 ml of ethyl acetate are added, and the mixture is washed with water (3 × 250 ml) and saturated sodium chloride solution. The organic phase is dried over magnesium sulphate, and the solvent is removed under reduced pressure. 1.81 g (42% of theory) of the desired product are obtained.

R_f = 0.24 (dichloromethane/methanol 100:2)

MS (ESI): m/z = 355 $[M+H]^+$

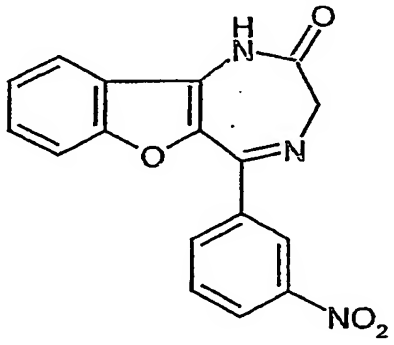
LC/MS (method 1): R_t = 3.47 min, m/z = 354 $[M]^+$

^{1}H-NMR (200 MHz, DMSO-d_6): δ = 4.45 (s, 2H); 7.37-7.80 (m, 6H); 7.88-7.93 (m, 1H); 7.96-8.03 (m, 1H); 11.59 (s, 1H).

The following are obtained in an analogous manner:

Example 2

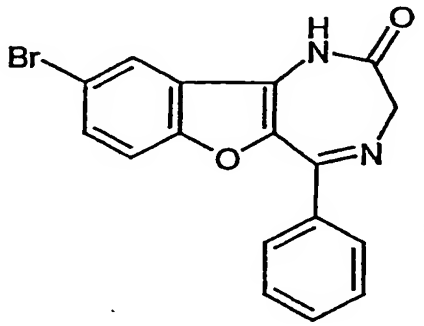
1,3-Dihydro-5-(3-nitrophenyl)-2*H*-benzofuro[3,2-e]-1,4-diazepin-2-one



LC/MS (method 2): R_t = 3.5 min, m/z = 321 $[M+H]^+$.

Example 3

9-Bromo-1,3-dihydro-5-phenyl-2*H*-benzofuro[3,2-e]-1,4-diazepin-2-one



LC/MS (method 2): R_t = 3.5 min, m/z = 355 $[M+H]^+$.

C. Description of the biological tests:

a) Cellular assay:

The $P2X_4$ receptor is a ligand-activated ion channel. Binding of the agonist ATP leads to activation of the $P2X_4$ receptor, opening of the ion channel and influx of the extracellular calcium into the cell. This calcium influx is measured with the aid of the calcium-sensitive photoprotein acquorin. For this purpose, a recombinant CHO cell line (Chinese hamster ovary cells) with constitutive expression of the human $P2X_4$ receptor and of apoaequorin was prepared.

The experiment is carried out by seeding the CHO cells 1-2 days beforehand onto microtitre plates in the 96-, 384- or 1536-well format, and specifically, in accordance with the microtitre plate format used, with 5000 (96 format), 2000 (384 format) or 500 (1536 format) cells per well. On the day of the experiment, the cell culture medium is removed and the cells are incubated with 5 µg/ml coelenterazine in physiological saline (Tyrode buffer) for 4 hours. Test substances are added 5 minutes before the actual experiment. The $P2X_4$ receptor is then activated by adding ATP in a concentration of 1-2 µm, and the ATP-induced calcium signal is measured as aequorin luminescence in a luminometer.

Substances with antagonistic activity on the $P2X_4$ receptor can inhibit the ATP-induced calcium signal either by interference with the binding of ATP to the $P2X_4$ receptor, by preventing channel opening or by blocking calcium influx through the opened channel.

Exemplary embodiments 1-3 show IC_{50} values of respectively 0.5, 2 and 0.6 µm in this test.

b) ATP-induced oxygen free radical formation (ROS) in primary human monocytes:

The assay is carried out in Hank's balanced salt solution (HBSS) to which 10 mM glucose is added. Monocytes are isolated for example by use of the "Becton Dickinson Vacutainer System" as described by the manufacturer, and suspended in HBSS. The oxygen free radicals are detected in principle by the luminol-enhanced chemiluminescence method in the presence of horse radish peroxidase (HRPO) [H. Lundqvist, C. Dahlgren, *Free Radic. Biol. Med.* 20 (6): 785-92 (1996)].

Firstly, the substance to be investigated, luminol (final concentration 50 µm), HRPO (final concentration 10 U/ml) are incubated with 5×10^5 monocytes at 37°C for 15 min. ATP is then added to the test mixture (final concentration 100 µM). The final volume in the test mixture is 200 µl. Immediately after addition of ATP, the ROS formation is followed using a microplate luminometer over a period of 120 seconds.

c) ATP-induced chemotaxis of primary human monocytes:

Monocytes are isolated from blood by standard methods. The chemotaxis of the monocytes is observed in a Transwell system [C.C. Bleul et al., *J. Exp. Med.* 184: 1101-1109 (1996)]. The membrane used (pore size 3 µm, polyethylene terephthalate, from Falcon) is initially coated with fibronectin. 10^5 monocytes in RPMI 1640 medium are put into the upper chamber. The lower chamber contains varying concentrations of stimulus or constant stimulus concentration (500 µM ATP or 10 nM MCP-1) and varying concentrations of the test substance. The substances to

be investigated are present in both chambers. The test mixture is incubated at 37°C with 5% CO_2 for 3 h [W. Falk et al., *J. Immunol. Methods* 38: 239-247 (1980)]. After the incubation, the cells which have migrated into the lower chamber are determined.

D. Exemplary embodiments of pharmaceutical compositions:

The compounds of the invention can be converted into pharmaceutical preparations in the following ways:

Tablet:

Composition:

100 mg of the compound of Example 1, 50 g of lactose (monohydrate), 50 mg of corn starch (native), 10 mg of polyvinylpyrrolidone (PVP 25) and 2 mg of magnesium stearate.

Tablet weight 212 mg. Diameter 8 mm, radius of curvature 12 mm.

Production:

A mixture of active ingredient, lactose and starch is granulated with a 5% strength solution (m/m) of the PVP in water. The granules are dried and then mixed with the magnesium stearate for 5 min. This mixture is compressed in a conventional tablet press (see above for format of the tablet). A compressive force of 15 kN is used as guideline for the compression.

Suspension which can be administered orally:

Composition:

1000 mg of the compound of Example 1, 1000 mg of ethanol (96%), 400 mg of Rhodigel® (xanthan gum from FMC, Pennsylvania, USA) and 99 g of water.

10 ml of oral suspension are equivalent to a single dose of 100 mg of the compound of the invention.

Production:

The Rhodigel is suspended in ethanol, and the active ingredient is added to the suspension. The water is added while stirring. The mixture is stirred for about 6 h until the swelling of the Rhodigel is complete.